PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

WRITTEN OPINION
(PCT Rule 66)

Date of mailing *(day/month/year)*	25.01.2005
Applicant's or agent's file reference: Sabanci PCT 3	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/TR 03/00019	20.03.2003	20.03.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/62

Applicant
SABANCI UNIVERSITESI et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 20.07.2005

Name and mailing address of the international preliminary examining authority:

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Seranski, P

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I. Basis of the opinion

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-35 as originally filed

Claims, Numbers

1-16 as amended (together with any statement) under Art. 19 PCT

Drawings, Sheets

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☒ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-5, 10
Inventive step (IS)	Claims	6-9, 11-16
Industrial applicability (IA)	Claims	1-16

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The application relates to a method of immobilization, visualisation and quantification of proteins on a support material. The application aims to provide alternative to conventional His-Tag-Ni-Cellulose purification techniques. However, claimed are vectors that comprise the same elements as already known in the prior art.

Reference is made to the following documents:

D1: WO 99 57992 A (CLONTECH LAB INC) 18 November 1999 (1999-11-18)

D2: DE 100 13 204 A (DEUTSCHES KREBSFORSCH) 11 October 2001 (2001-10-11)

D3: CHA HYUNG JOON ET AL: "Observations of green fluorescent protein as a fusion partner in genetically engineered Escherichia coli: Monitoring protein expression and solubility" BIOTECHNOL BIOENG;BIOTECHNOLOGY AND BIOENGINEERING 2000 JOHN WILEY & SONS INC, NEW YORK, NY, USA, vol. 67, no. 5, 2000, pages 565-574,

D4: KEEFE ANTHONY D ET AL: "One-step purification of recombinant proteins using a nanomolar-affinity streptavidin-binding peptide, the SBP-tag." PROTEIN EXPRESSION AND PURIFICATION, vol. 23, no. 3, December 2001 (2001-12), pages 440-446, ISSN: 1046-5928

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-5 is not new in the sense of Article 33(2) PCT.

Document D1 provides vector constructs comprising Green Fluorescent Protein, a Multiple Cloning Site and an affinity peptide. The affinity peptide aims for the purification of the protein that is to be expressed by the vector construct (See.Fig.1). Said affinity peptide is specifically mentioned to be a histidine-rich polypeptide sequence.

Also document D2-D4 provide for vector constructs with a visual marker protein like

GFP, a multiple cloning site, and protein tags like a His-tag or a streptavidin binding protein. All disclosed vector constructs have the property that they can produce fusion-protein that can be further immobilized, visualized and quantified. Consequently, documents D1-D4 all destroy the novelty of the product claims 1-5.

Dependent claims 6-9 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Art.33(3) PCT)

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 10 is not new in the sense of Article 33(2) PCT.

Document D4 discloses a construct as described supra, said construct is used in method of purification of a protein. The document explicitly refers to the streptavidin binding protein that can be used for detection of the recombinant protein for example in a matrix system like microtiter-plates. The streptavidin tagged protein can also be quantified as shown in the document for methods for the measurement protein-protein, protein-peptide or protein-small molecule equilibrium dissociation constants. All characterising features of claim 10 can thus be found in D4, the engineering of the construct (step a), inserting the gene of the target protein in the MCS (step b), protein expression (step c) as well as the expression and the immobilisation and washing (e-f). Steps d, g and h are optional and have therefore no limiting effect to the claimed method. Therefore the method of claim 10 is not new (Art.33(2) PCT).

Dependent claims 11-16 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Art.33(3) PCT)